IOP Conference Series: Materials Science and Engineering

PAPER • OPEN ACCESS

Detection and recognition of micro-nano fluorescent particle array based on Alex-Net

To cite this article: Y Y Zhang *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **504** 012035

IOP Conf. Series: Materials Science and Engineering **504** (2019) 012035 doi:10.1088/1757-899X/504/1/012035

Detection and recognition of micro-nano fluorescent particle array based on Alex-Net

Y Y Zhang[1], Z W Cheng[2], Y C Jin[3], K Si[4] and X Y Jin[5,*]

[1]State Key Laboratory of Modern Optical Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou, 310027, China
[2,3,5]Institute of Information Science and Electronic Engineering, Zhejiang University, Hangzhou 310058, China
[4]Center for Neuroscience, Department of Neurobiology, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou, 310058, China

Corresponding author and e-mail: X Y Jin, jinxy@zju.edu.cn

Abstract. Micro-nano fluorescent particle functioning as a kind of detection material with ultra-high sensitivity are widely applied to various fields and have wide application prospects. When excited by external energy, the fluorescent particle emits which can be detected by optical detector with high sensitivity. However, the scattering media will introduce severe aberration which decreases the quality of the fluorescent images. Furthermore, when detecting the fluorescent particle array with spatial pattern, the aberration leads to more unrecognizable images. This paper proposes a method of detecting and recognizing micro-nano fluorescent particle array based on deep learning, which achieved detection and recognition with the precision rate of 97.29%.

1. Introduction

Micro-nano fluorescent particle is a kind of fluorescent material, the diameter of which is in the micron or nanometer scale. Compared with conventional fluorescent probes or organic fluorescent dyes, they are able to emit strong enough characteristic fluoresce and maintain the stability of the signal. As a detection material with high sensitivity, the micro-nano fluorescent particle is applied to various application fields.

In the field of biological research, the fluorescent particle has been successfully applied to probing motion in living cells and cell structure changes in biological system [1], which plays an important role in observation of organisms and tracking of biological phenomena. Fluorescent particle can also be applied to measuring the flow and composition of fluid-solid systems in micro-fluidic devices [2] and investigating the fluidic and other properties relative to the particle distribution in solid-liquid suspensions [3] by being tracked in three dimensions.

The observation of fluorescent particle is based on imaging system with ultra-high sensitivity. However, imaging instruments are designed to achieve optimal performance in samples with specific optical properties [4]. When the scattering media is unpredictable, it changes the direction and phase of the light rays and distorts the wavefront of the light from the ideal spherical form [4]. Luminance, contrast ratio and quality of image will decrease sharply.

At present, Luo *et al.* proposed that the diffraction pattern with several clear interference fringes generated from the fluorescence emitted from a fluorescent particle changes with the particle's position along the axis in [5], which can be used to track fluorescent particle. F.Chasles *et al.* presented a technique for imaging fluorescent particle based on the axial modulation of the objective's focal plane position in [6] which provides full-field optical sectioning and can be used to localize the fluorophores in three dimensions [6]. However, it is more challengeable to achieve accurate detection and recognition of fluorescent particle array with specific spatial pattern which is used to mark samples or analyze the deformation of samples. The spatial information of the particle array is damaged due to the aberration, which leads to failures of detection.

In this paper, we introduce the well-developed Alex-Net neural networks and get dataset to train it by the optical simulation system. As a result, we have achieved accurate detection and recognition with precision rate of 97.29%.

2. Observation system of the fluorescent particle array

In this paper, the fluorescent particles are observed by a wide field microscopy, whose optical system is shown in Figure 1. (a). A 632.8nm laser beam is used as excitation light. The fluorescence emitted by fluorescent particle is emission light which is detected by a CMOS at the conjugated focal plane. Figure 1 (b) and (c) are fluorescent images before and after scattering.

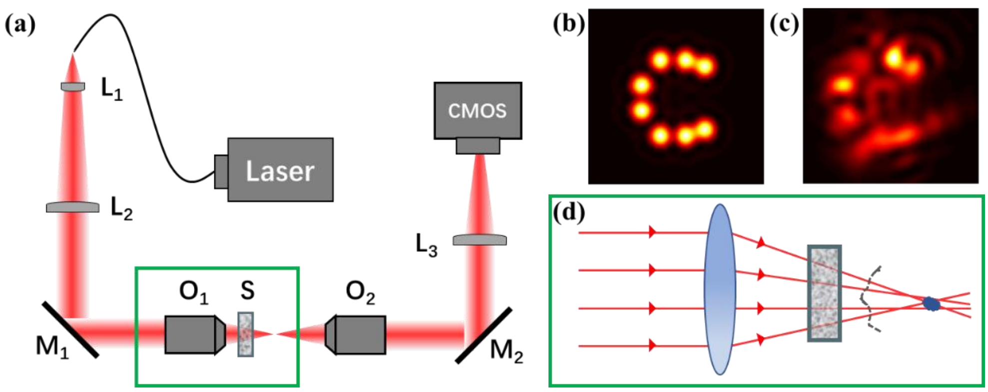

Figure 1. Principle of optical simulation system. (a) Schematic of optical system. A laser beam is firstly expended by relay lens L1 and L2. Then it transforms through objective lens O1 and scattered by sample S. Finally, the scatted fluorescent image is collected by objective lens O2 and detected by the CMOS. (b) Eight fluorescent particles forming a specific pattern (A-J) without scattering. (c) Distorted fluorescent image with scattering. (d) is the simplified model of the optical system (green box) in (a).

3. Detection and recognition of fluorescent particle array based on deep learning

In this paper, we simulated an optical system and produced a large number of images with fluorescent particle array distorted by digital scattering media. Alex-Net neural network is adopted as learning framework.

3.1. Optical system simulation

The simplified simulation model of the optical system presented here consists of multiple steps, which are visualized in Figure 1. (d). It is equal to the optical path illustrated within green boxes in Figure 1. (a), which consists of two parts mainly, the objective lens and the scattering media. The scattering media is simulated with a composition of 15 layers scattering phantom.

In order to generate a large quantity of distorted fluorescent images, we simulate a parallel light as an incident beam which transforms through a lens and focusing into the focal plane after scattering.

Due to the thickness variation of the lens, it has a phase modulation on the incident beam which can be expressed as:

$$t_l(x,y) = \exp[-j\frac{k}{2f}(x^2+y^2)] \tag{1}$$

where t_l is the phase modulation introduced by lens, k is the wave vector, f is the focal length of the lens. x and y are coordinates.

As for the phase modulation induced by the scattering media, we can calculate the field strength at the focal plane according to the diffraction angular spectrum theory. The core equation is:

$$A\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right) = A_0\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right)exp(jkz\sqrt{1-\cos^2\alpha-\cos^2\beta}) \tag{2}$$

where $A\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right)$ is new angular spectrum of the initial plane after old angular spectrum $A_0\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right)$ of the objective plane transforms a distance of z. α represents the angle between the k-vector and the x axis and β represents the angle between the k-vector and the y axis.

When the transform direction satisfy $\cos^2\alpha - \cos^2\beta \ll 1$, it can be approximately expressed as:

$$A\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right) = A_0\left(\frac{\cos\alpha}{\lambda},\frac{\cos\beta}{\lambda}\right)exp(jkz) \tag{3}$$

The old angular spectrum of the objective plane can be obtained by Fourier transform of the field strength. Transform distance z only introduces a certain phase shift, and the amplitude is not affected. The new angular spectrum is then inversely transformed by Fourier transform to obtain the new light field distribution at the objective plane.

3.2. Alex-Net deep convolutional neural network

In the field of computer vision, Alex-Net is the first convolutional neural network that has been widely focused and applied. The neural network has five convolution layers, some of them are followed by the maxpool layer, and ends up with three fully connected layers of 10 characters classification using softmax function. In this paper, the network structure is shown in Figure 2 based on the classic Alex-Net network model [7].

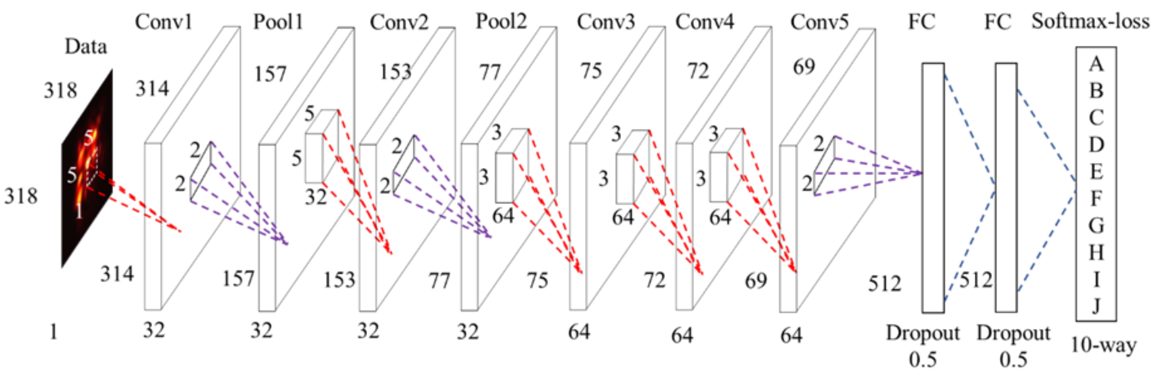

Figure 2. Schematic of Alex-Net deep convolutional neural works.

Nair and Hinton introduced Rectified Linear Unit (ReLU) to neural networks to avoid the happening of gradient saturation phenomenon in 2010 [8]. ReLU is one of the most commonly used activation functions in deep neural networks and is a piecewise function (ReLU(x) = max{0,x}) calculated easily. It is also found in experiment that the convergence speed of stochastic gradient descent is about 6 times faster [7].

Dropout is the regularization method used widely in deep neural networks equipped with full-connected layers, which aims to improve generalization capability [9]. It will set to zero the output of each hidden neuron with probability p. The neurons which are "dropped out" in this way do not contribute to the forward pass and do not participate in back-propagation. So every time an input is presented, the neural network samples a different architecture, but all these architectures share weights. [7] Dropout eases the complex synergistic effect between neurons and reduces the interdependencies between neurons. In this work, we set the p as 0.5.

4. Results and discussion

11,000 labelled images with distortion are generated through simulation model. We consider that the fluorescent particle will rotate due to different relative position between imaging system and the samples. As a result, in this paper, each image in the dataset is randomly rotated with a certain degree, which is closer to the real condition. Among them, 10,000 data sets are randomly divided into training set and validation set according to the ration of 3:1 and the rest of them are set as test set. The results of the experiment are shown in Table 1, which displays the precision, recall and F_1_score of detecting and recognizing distorted images. Precision (P) represents the differentiation ability of the model to negative samples. Recall (R) represents the recognition ability of the model to positive samples. F_1_score represents the stability of the model. The results show that we have achieved detection and recognition of fluorescent particle with the precision rate of 97.29% on test set.

Table 1. Results of the experiment.

	A	B	C	D	E	F	G	H	I	J
P	0.984	0.971	0.988	0.895	0.991	0.989	0.993	0.990	0.978	0.950
R	0.955	0.964	0.990	0.987	0.962	0.986	0.953	0.999	0.981	0.981
F_1_score	0.969	0.967	0.989	0.939	0.976	0.987	0.973	0.994	0.979	0.965

12 groups of experimental results are shown in Figure 3. (a). Each group consists of two pictures (representing ideal image and distorted image respectively) and the predicted result of the distorted image. The most of distorted images can be recognized accurately by trained Alex-Net neural networks. However, the distorted images of similar patterns will be predicted wrongly, such as the distorted image of "I" is wrongly predicted to be "J". It is shown in Figure 3. (b), which is discussed based on the individual imaging of each fluorescent particle. Figure 3. (c) & (d) represent the images of single fluorescent particle in green box and blue box of Figure 3. (b) respectively. We find that the intensity of the image can be influenced irregularly by the scattering media. The intensity of (c) is obvious lower than that of (d), so that the image of fluorescent particle in the green box in (b) will be ignored easily. The images of red box in (b) are shown in Figure 3. (e) & (f). We find that fluorescent particle in red box which is close to each other are judged to be only one particle. It is because the distorted images of them overlap to some degree. These matter leads to the difficulty in detection and recognition of fluorescent particle array. The proposed method based on deep learning avoids making specific these detection criteria and dealing with each particle separately.

5. Conclusions

Fluorescent particles have wide application prospects in the research field. However the scattering media will introduce severe aberration which distorts the fluorescent images. We introduce well-developed Alex-Net neural network to detect and recognize fluorescent particle array and achieve outstanding accuracy. In future work, capturing real images is an efficient way to obtain dataset for real condition. Furthermore, introducing various complex interference sources can improve the practicality and robustness of the proposed method.

Acknowledgement
This work was supported by the opening foundation of the State Key Laboratory (No.2014KF06), and the National Science and Technology Major Project (No.2013ZX03005013).

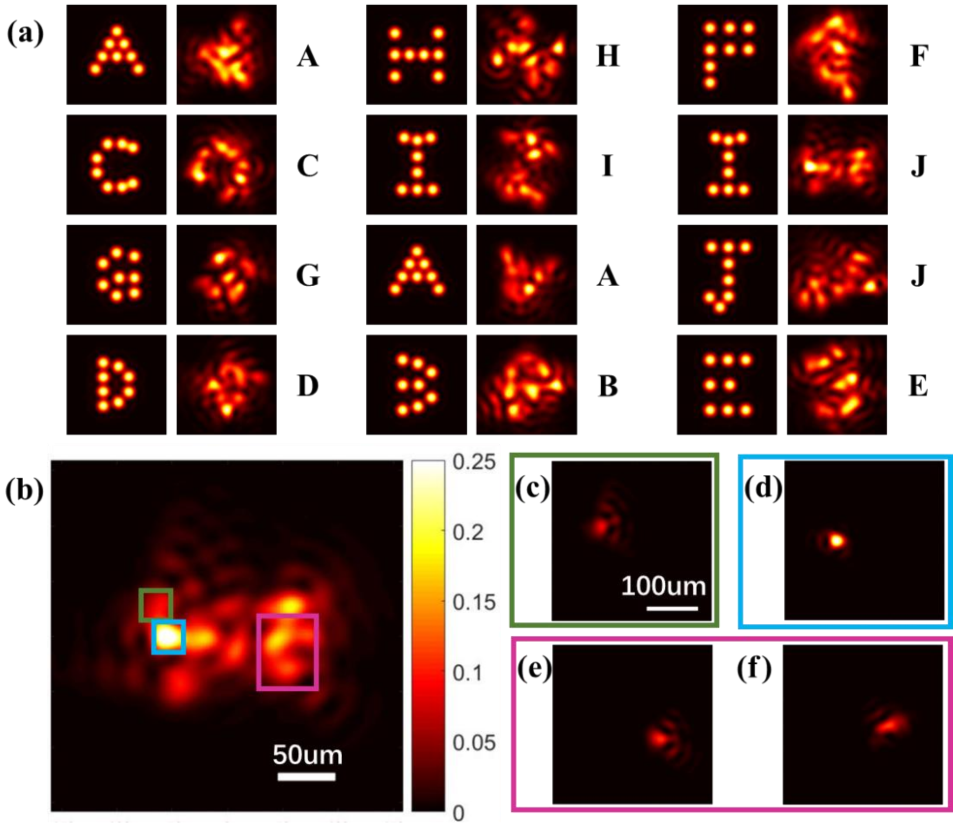

Figure 3. Demonstration and analysis of experimental results. (a) displays 12 groups of experimental results, which consist of ideal image, distorted image and predicted results. (b). A distorted image. The single fluorescent particle in green box is shown in (c). Another fluorescent particle in blue box is shown in (d). (e) and (f) are separate images of the two fluorescent particles in the red box.

References
[1] Caspi A, Granek R, Elbaum M. 2002 *J. Physical Review E*. **66** 011916.
[2] Wu M M, Roberts J W and Buckley M. 2005 *J.Exp. Fluids*. **38** 461-65
[3] Schroeder C M, Shaqfeh E S G and Chu S 2004 *J.Macromolecules*. **37** 9242-56
[4] Na Ji. 2017 *J.Nature Methods*. **14** 374-80.
[5] R Luo, X Y Yang, X F Peng and Y F Sun 2006 *J.Micromech.Microeng*. **16** 1689-99
[6] F.Chasles, B.Dubertret and A.C.Boccara 2006 *J.Optics.Letters*. **31** 1274-76
[7] Alex Krizhevsky, Ilya Sutskever, and Geoffery E.Hinton. 2012 *A. International Conference on Neural Information Processing Systems*. 1097-105
[8] Vinod Nair and Geoffrey E.Hinton. 2010 *A.In Proceedings of International Conference on Machine Learning*. 807-14
[9] Hinton G E, Srivastava N, Krizhevsky A, et al. 2012 *J.Computer Science*. **3** 212-23